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Semi-annual Progress Report

Title of Project:

An investigation of the mechanisms of oxidatove coupled phosphorylation in bacterial extracts.

Period reported on:

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Name of Principle Investigator:

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Name of Contractor:

Yale University

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In the previous reports studies on coupled phosphorylation in soluble extracts of E. coli were described. Energy rich phosphate formed in the oxidation of reduced DPN by these extracts was trapped as hexose phosphates by means of the hexokinase reaction. The coli extracts, however, have an active glycolytic pathway by which these hexose phosphates can be further metabolized with the formation of additional energy rich phosphate. In order to eliminate this confusion side reaction, it was necessary to treat the coli extracts with iodoacetate and sodium fluoride to inhibit glycolysis. The formation of reduced DPN in the reaction mixture with alcohol, alcohol dehydrogenase, and DPN introduced another complication, since the coli extracts oxidized the acetaldehyde formed in this reaction to acetyl phosphate, with a further yield of high energy phosphate. This was prevented by removing the required Coenzyme A from the extracts with Dowex.

Because of these complicating reactions, and the danger that the inhibitors used to prevent them might also inhibit coupled phosphorylation, it was decided to work with a bacterium known not to ferment glucose. Alkaligenes fecalis was chosen and proved to be completely satisfactory, since extracts of this organism carried out oxidative phosphorylation, but would not metabolize glucose-6 phosphate, hexose diphosphate or acetaldehyde. Energy rich phosphate was trapped with the hexokinase reaction and determined either as inorganic phosphate disappearance, or more frequently as accumulation of glucose-6 phosphate. The latter determination was done spectrophotometrically using either glucose-6 phosphate dehydrogenase or alpha glycerophosphate dehydrogenase and the appropriate enzymes to link this to hexose monophosphate.

When alcohol, alcohol dehydrogenase, and DPN were used as the hydrogen

donor system, 2 to 3 micromoles of glucose-6 phosphate were recovered with P/O ratios over 1 in some cases. Sodium fluoride was strongly inhibitory to phosphate esterification, while semi-carbazide (5×10^{-2} M.) was somewhat less inhibitory, and dinitrophenol, in the concentration used to inhibit animal tissues, had no effect. It was found that increasing the inorganic phosphate concentration from 10 to 250 micromoles per vessel increased the P/O ratios, and that the use of ADP rather than ATP produced better yields of high energy phosphate.

With the alkaligenes extracts it was possible to demonstrate phosphorylation associated with the oxidation of preformed DPNH (no alcohol or alcohol dehydrogenase added to the reaction mixture). This was never possible with the E. coli extracts. The ratios were below 0.5 and the total yield of phosphorus 1 micromole or below.

These findings help to explain some of the previous difficulties with the coli experiments. Suboptimal concentrations of phosphate and ADP, plus the inhibitory effect of sodium fluoride would provide ample reason for the difficulties encountered. Since the Alkaligenes extracts posses the advantages of withstanding high speed centrifugation, freezing and thawing, and ammonium sulphate fractionation, it is hoped that further experiments will permit a thorough understanding of the mechanisms of oxidative phosphorylation in these bacteria.

Housekeeping details remain essentially unchanged. Mr. Kern, a graduate student, is continuing his work on DPNH oxidase. We have a part time glass washer, John Arnez.

No support from other sources is being received for this project, nor has any been applied for.